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FIG. 1

SEQ ID NO: 1:

GAGAAGGTTT GTTATGCCTC AGGGTTATCT GCAGTTTCCC AATATTGACC CCGTATTGTT 60
TTCGATCGGC CCTCTAGCGG TCGCTGGTA TGGCTTGATG TATTTGGTGG GTTTCCTTTT 120
TGCTATGTGG TTGGCCAATC GCCGAGCGGA TCGCGCGGGC AGTGGTTGGA CGCGTGAGCA 180
AGTCTCTGAC TTGTTATTCC CCGGCTTTTT AGGTGTAGTG ATCGGTGGCC GAGTTGGTTA 240
TGTGATCTTC TACAATTTTG ATCTGTTCTT TGCTGACCCT CTTTATTTAT TCAAAGTGTG 300
GACTGGCGGC ATGTCCTTCC ACGGCGGCTT ATTGGGTGTG ATCACCGCCA TGTTCCTGGTA 360
TGC GCGTAAA AACCAACGCA CCTTCTTTGG TGTGGCCGAT TTTGTTGCCC CTTTAGTGCC 420
ATTCGGTTTG GGGATGGGAC GTATCGGTAA CTTTATGAAT AGTGAACCTT GGGGACGAGT 480
AACGGATGTG CCTTGGGCTT TTGTATTCCC TAATGGTGGC CCACTGCCGC GCCATCCTTC 540
ACAGCTTTAT GAATTCGCCT TAGAAGGCGT GGTTCGTTC TTTATTCTTA ATTGGTTTAT 600
TGGTAAACCT CGTCCGCTAG GCAGCGTATC CGGACTGTTT TTAGCTGGAT ACGGTACATT 660
CCGCTTCCTT GTGGAATACG TCCGTGAGCC AGATGCTCAG TTGGGTCTGT TTGGTGGCTT 720
CATTTCAATG GGGCAAATCC TCTCCTTACC TATGGTGATC ATCGGTATTT TGATGATGGT 780
TTGGTCTTAC AAGCGCGGTT TGTATCAAGA CCGTGTAGCA GCAAAATAGG GTAGTTAGGT 840
GAAACAGTAT TTAGATCTTT GTCAGCGCAT CGTCGATCAA GGTGTTTGGG TTGAAAATGA 900
ACGAACGGGC AAGCGTTGTT TGA CTGTGAT TAATGCCGAT TTGACCTACG ATGTGGGCAA 960
CAATCAGTTT CCTCTAGTGA CTACACGCAA GAGTTTTTGG AAAGCTGCCG TAGCCGAGTT 1020
GCTCGGCTAT ATTCGTGGTT ACGATAATGC GCGGATTTT CGCCAATTAG GTACCAAAC 1080
CTGGGATGCT AATGCCAATT TAAACCAAGC ATGGCTCAAC AATCCTTACC GTAAAGGTGA 1140
GGATGACATG GGACGCGTGT ATGGTGTTC A GGTAGAGCT TGGGCTAAGC CTGATGGTGG 1200
TCATATTGAC CAGTTGAAAA AGATTGTTGA TGATTTGAGC CGTGGCGTTG ATGACCGAGG 1260
TGAAATTCTT AACTTCTACA ATCCGGGTGA ATTTACATG GGGTGTTCG GCCCTTGCAT 1320
GTACAGCCAT CATTTTTTCAT TGCTGGGGGA TACCTTGAT CTCAACAGTA CTCAGCGTTC 1380
ATGTGATGTG CCCTTGGGGT TGAATTTCAA CATGGTGCAG GTTTATGTGT TCCTTGCGCT 1440
GATGGCACAG ATCACAGGA AAAAGCCGGG CTTGGCGTAT CACAAGATCG TCAATGCGCA 1500

FIG.1 (cont.)

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CATTACCAA GATCAACTCG AATTGATGCG CGATGTGCAG CTAAAACGTG AGCCATTCCC 1560
AGCGCCTCAG TTCCATATCA ATCCAAAGAT TAAAACACTG CAGGATTTGG AACTTTGGGT 1620
CACTTTGGAT GATTTTGACG TCACCGGATA TCAGTTCCAC GATCCTATTC AATACCCGTT 1680
TTCAGTCTAA TCCCGTATTC AGGCGGTATG GCTTGATGGG TTTTATATAA AAAAAGCTCC 1740
CGAAGGTCGG GAGCTTTTTT TATACAGATG ATGCTTTAAC GCTTAAGCGG TTAGGGCAAG 1800
AATGCTGCCG GGGATGACGA CAAACACACC CAATAAGTAA CTCACCACCA CCATTTTGCT 1860
CTTACAAGCC CAAGTTGAGA TGAGCTCAGC ACCTTTAATA GGCAGTTCGC GTAAGAAAGG 1920
AATACCGTAA ATCAAGACCG TAGCCATCAA GTTAAAGCTT AAGTGCACCA GCGCAATTG 1980
CAGAGCAAAC ACGGCAAAC CACCAGAGAC AGCGGTTGCG GCGAGCAGAG CAGTAATACA 2040
AGTGCCAATG TTCGCACCTA AGGTAAATGG GTAGATTTCA CGCACTTTCA GCACGCCAGA 2100
GCCCACGAGA GGAACCATTA GGCTGGTTGT GGTCGATGAA GATTGAACTA ATACCGTAAC 2160
CACTGTACCT GAAGCAATAC CGTGTAGTGG GCCTCGGCCA ATCGCATTTT GTAGAATTTT 2220
ACGTGCGCGG CCAACCATCA AACTCTTCAT CAGTTTGCCC ATCACCGTAA TGGCGACGAA 2280
AATGGTCGCA ATACCCAATA CGATAAGTGC GACACCACCG AAAGTATTAC CCAATACCGA 2340
AAGCTGGGTT TCAAGCCCTG TGATGACAGG TTTGGTAATC GGTTTGATAA AATCAAAACC 2400
TTTCATGCTC ATATCGCCAG TCGCAAGCAG AGGCGAAACG AGCCAGTGTG AGACTTTCTC 2460
TAAAATGCCA AACATCATTT CTAGAGGTAG GAAGATCAGC ACCGCGAGAA GATTGAAAAA 2520
ATCGTGGATG GTGGCACTGG CGAAAGCAGG GCGAAACTCT TCTTTACAGC GCATATGGCC 2580
AAGGCTGACG AGAGTATTGG TCACAGTAGT ACCAATATTG GCACCCATCA CCATAGGAAT 2640
CGCGGTTTCA ACCGGTAACC CACCGGCAAC GAGACCAACA ATAATAGAAG TCACCGTGCT 2700
TGAGGATTGA ATCAGTGCCG TTGCCACTAA ACCAATCATC AATCCTGCAA TTGGGTGGGA 2760
AGCAAATTCA AATAGAACTT TGGCTTGATC GCCGGTTGCC CATTTAAAC CGCTGCCGAC 2820
CATCGCGACT GCAAGAAGTA GTAAATACAG CATGAAAGCC AAGTTTGCCC AACGTAGGCC 2880
TTTCGTGGTC AGCGAAATCG GCGCTGCAG 2909

FIG.2

SEQ ID NO: 2:

GAGAAGGTTT GTTATGCCTC AGGGTTATCT GCAGTTTCCC AATATTGACC CCGTATTGTT 60
TTCGATCGGC CCTCTAGCGG TCGCTGGTA TGGCTTGATG TATTTGGTGG GTTTCCTTTT 120
TGCTATGTGG TTGGCCAATC GCCGAGCGGA TCGCGCGGGC AGTGGTTGGA CGCGTGAGCA 180
AGTCTCTGAC TTGTTATTCG CCGGCTTTTT AGGTGTAGTG ATCGGTGGCC GAGTTGGTTA 240
TGTGATCTTC TACAATTTTG ATCTGTTCTT TGCTGACCCT CTTTATTTAT TCAAAGTGTG 300
GACTGGCGGC ATGTCCTTCC ACGGCGGCTT ATTGGGTGTG ATCACCGCCA TGTTCCTGGTA 360
TGCGCGTAAA AACCAACGCA CCTTCTTTGG TGTGGCCGAT TTTGTTGCCC CTTTAGTGCC 420
ATTCGGTTTG GGGATGGGAC GTATCGGTAA CTTTATGAAT AGTGAACTTT GGGGACGAGT 480
AACGGATGTG CCTTGGGCTT TTGTATTCCC TAATGGTGGC CCACTGCCGC GCCATCCTTC 540
ACAGCTTTAT GAATTCGCCT TAGAAGGCGT GGTTCTGTTC TTTATTCTTA ATTGGTTTAT 600
TGGTAAACCT CGTCCGCTAG GCAGCGTATC CGGACTGTTT TTAGCTGGAT ACGGTACATT 660
CCGCTTCCTT GTGGAATACG TCCGTGAGCC AGATGCTCAG TTGGGTCTGT TTGGTGGCTT 720
CATTTCATG GGGCAAATCC TCTCCTTACC TATGGTGATC ATCGGTATTT TGATGATGGT 780
TTGGTCTTAC AAGCGCGGTT TGTATCAAGA CCGTGTAGCA GCAAAATAGG GTAGTTAG 838

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FIG.3

SEQ ID NO: 3:

TAATCCCGTA TTCAGGCGGT ATGGCTTGAT GGGTTTTATA TAAAAAAGC TCCCGAAGGT 60
CGGGAGCTTT TTTTATACAG ATGATGCTTT AACGCTTAAG CGGTTAGGGC AAGAATGCTG 120
CCGGGGATGA CGACAAACAC ACCCAATAAG TAACTCACCA CCACCATTTT GCTCTTACAA 180
GCCCAAGTTG AGATGAGCTC AGCACCTTTA ATAGGCAGTT CGCGTAAGAA AGGAATACCG 240
TAAATCAAGA CCGTAGCCAT CAAGTTAAAG CTTAAGTGCA CCAGCGCAAT TTGCAGAGCA 300
AACACGGCAA ACTCACCAGA GACAGCGGTT GCGGCGAGCA GAGCAGTAAT ACAAGTGCCA 360
ATGTTTCGCAC CTAAGGTAAA TGGGTAGATT TCACGCACTT TCAGCACGCC AGAGCCCACG 420
AGAGGAACCA TTAGGCTGGT TGTGGTCGAT GAAGATTGAA CTAATACCGT AACCCTGTGA 480
CCTGAAGCAA TACCGTGTAG TGGGCCTCGG CCAATCGCAT TTTGTAGAAT TTCACGTGCG 540
CGGCCAACCA TCAAACCTCTT CATCAGTTTG CCCATCACCG TAATGGCGAC GAAAATGGTC 600
GCAATACCCA ATACGATAAG TGCACACCA CCGAAAGTAT TACCCAATAC CGAAAGCTGG 660
GTTTCAAGCC CTGTGATGAC AGGTTTGGTA ATCGGTTTGA TAAAATCAAA ACCTTTTCATG 720
CTCATATCGC CAGTCGCAAG CAGAGGCGAA ACGAGCCAGT GTGAGACTTT CTCTAAAATG 780
CCAAACATCA TTTCTAGAGG TAGGAAGATC AGCACCGCGA GAAGATTGAA AAAATCGTGG 840
ATGGTGGCAC TGGCGAAAGC ACGGCGAAAC TCTTCTTTAC AGCGCATATG GCCAAGGCTG 900
ACGAGAGTAT TGGTCACAGT AGTACCAATA TTGGCACCCA TCACCATAGG AATCGCGGTT 960
TCAACCGGTA ACCCACCAGC AACGAGACCA ACAATAATAG AAGTCACCGT GCTTGAGGAT 1020
TGAATCAGTG CCGTTGCCAC TAAACCAATC ATCAATCCTG CAATTGGGTG GGAAGCAAAT 1080
TCAAATAGAA CTTTGGCTTG ATCGCCGGTT GCCCATTTAA AACCGCTGCC GACCATCGCG 1140
ACTGCAAGAA GTAGTAAATA CAGCATGAAA GCCAAGTTTG CCCAACGTAG GCCTTTCGTG 1200
GTCAGCGAAA TCGGCGCTGC AG 1222

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FIG.4

SEQ ID NO: 4:

Val Lys Gln Tyr Leu Asp Leu Cys Gln Arg Ile Val Asp Gln Gly Val
 1 5 10 15
 Trp Val Glu Asn Glu Arg Thr Gly Lys Arg Cys Leu Thr Val Ile Asn
 20 25 30
 Ala Asp Leu Thr Tyr Asp Val Gly Asn Asn Gln Phe Pro Leu Val Thr
 35 40 45
 Thr Arg Lys Ser Phe Trp Lys Ala Ala Val Ala Glu Leu Leu Gly Tyr
 50 55 60
 Ile Arg Gly Tyr Asp Asn Ala Ala Asp Phe Arg Gln Leu Gly Thr Lys
 65 70 75 80
 Thr Trp Asp Ala Asn Ala Asn Leu Asn Gln Ala Trp Leu Asn Asn Pro
 85 90 95
 Tyr Arg Lys Gly Glu Asp Asp Met Gly Arg Val Tyr Gly Val Gln Gly
 100 105 110
 Arg Ala Trp Ala Lys Pro Asp Gly Gly His Ile Asp Gln Leu Lys Lys
 115 120 125
 Ile Val Asp Asp Leu Ser Arg Gly Val Asp Asp Arg Gly Glu Ile Leu
 130 135 140
 Asn Phe Tyr Asn Pro Gly Glu Phe His Met Gly Cys Leu Arg Pro Cys
 145 150 155 160
 Met Tyr Ser His His Phe Ser Leu Leu Gly Asp Thr Leu Tyr Leu Asn
 165 170 175
 Ser Thr Gln Arg Ser Cys Asp Val Pro Leu Gly Leu Asn Phe Asn Met
 180 185 190
 Val Gln Val Tyr Val Phe Leu Ala Leu Met Ala Gln Ile Thr Gly Lys
 195 200 205
 Lys Pro Gly Leu Ala Tyr His Lys Ile Val Asn Ala His Ile Tyr Gln
 210 215 220
 Asp Gln Leu Glu Leu Met Arg Asp Val Gln Leu Lys Arg Glu Pro Phe
 225 230 235 240
 Pro Ala Pro Gln Phe His Ile Asn Pro Lys Ile Lys Thr Leu Gln Asp
 245 250 255
 Leu Glu Thr Trp Val Thr Leu Asp Asp Phe Asp Val Thr Gly Tyr Gln
 260 265 270
 Phe His Asp Pro Ile Gln Tyr Pro Phe Ser Val
 275 280

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FIG. 5

SEQ ID NO: 5:

Met Pro Gln Gly Tyr Leu Gln Phe Pro Asn Ile Asp Pro Val Leu Phe
1 5 10 15

Ser Ile Gly Pro Leu Ala Val Arg Trp Tyr Gly Leu Met Tyr Leu Val
20 25 30

Gly Phe Leu Phe Ala Met Trp Leu Ala Asn Arg Arg Ala Asp Arg Ala
35 40 45

Gly Ser Gly Trp Thr Arg Glu Gln Val Ser Asp Leu Leu Phe Ala Gly
50 55 60

Phe Leu Gly Val Val Ile Gly Gly Arg Val Gly Tyr Val Ile Phe Tyr
65 70 75 80

Asn Phe Asp Leu Phe Leu Ala Asp Pro Leu Tyr Leu Phe Lys Val Trp
85 90 95

Thr Gly Gly Met Ser Phe His Gly Gly Leu Leu Gly Val Ile Thr Ala
100 105 110

Met Phe Trp Tyr Ala Arg Lys Asn Gln Arg Thr Phe Phe Gly Val Ala
115 120 125

Asp Phe Val Ala Pro Leu Val Pro Phe Gly Leu Gly Met Gly Arg Ile
130 135 140

Gly Asn Phe Met Asn Ser Glu Leu Trp Gly Arg Val Thr Asp Val Pro
145 150 155 160

Trp Ala Phe Val Phe Pro Asn Gly Gly Pro Leu Pro Arg His Pro Ser
165 170 175

Gln Leu Tyr Glu Phe Ala Leu Glu Gly Val Val Leu Phe Phe Ile Leu
180 185 190

Asn Trp Phe Ile Gly Lys Pro Arg Pro Leu Gly Ser Val Ser Gly Leu
195 200 205

Phe Leu Ala Gly Tyr Gly Thr Phe Arg Phe Leu Val Glu Tyr Val Arg
210 215 220

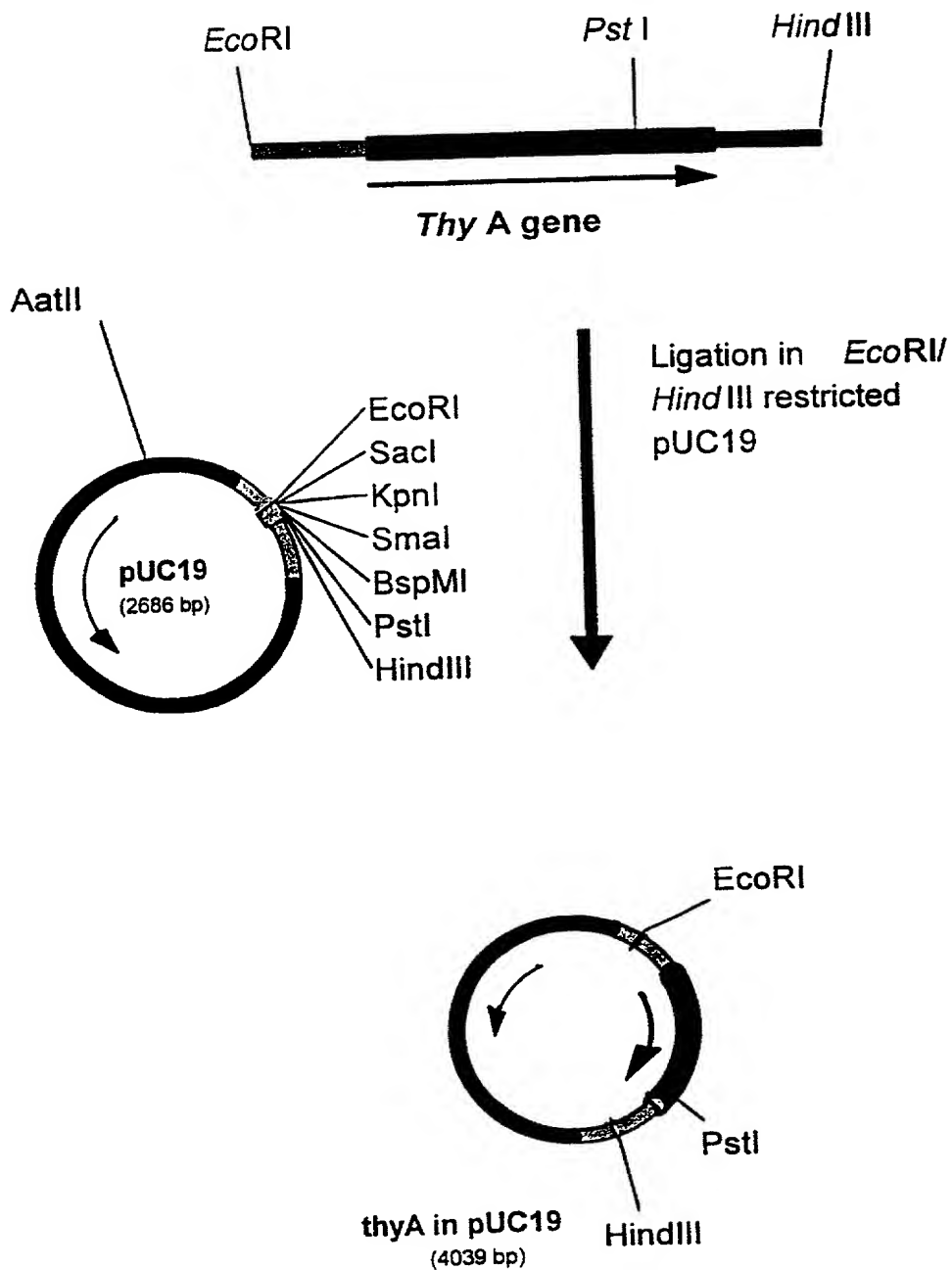
Glu Pro Asp Ala Gln Leu Gly Leu Phe Gly Gly Phe Ile Ser Met Gly
225 230 235 240

Gln Ile Leu Ser Leu Pro Met Val Ile Ile Gly Ile Leu Met Met Val
245 250 255

Trp Ser Tyr Lys Arg Gly Leu Tyr Gln Asp Arg Val Ala Ala Lys
260 265 270

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FIG. 6



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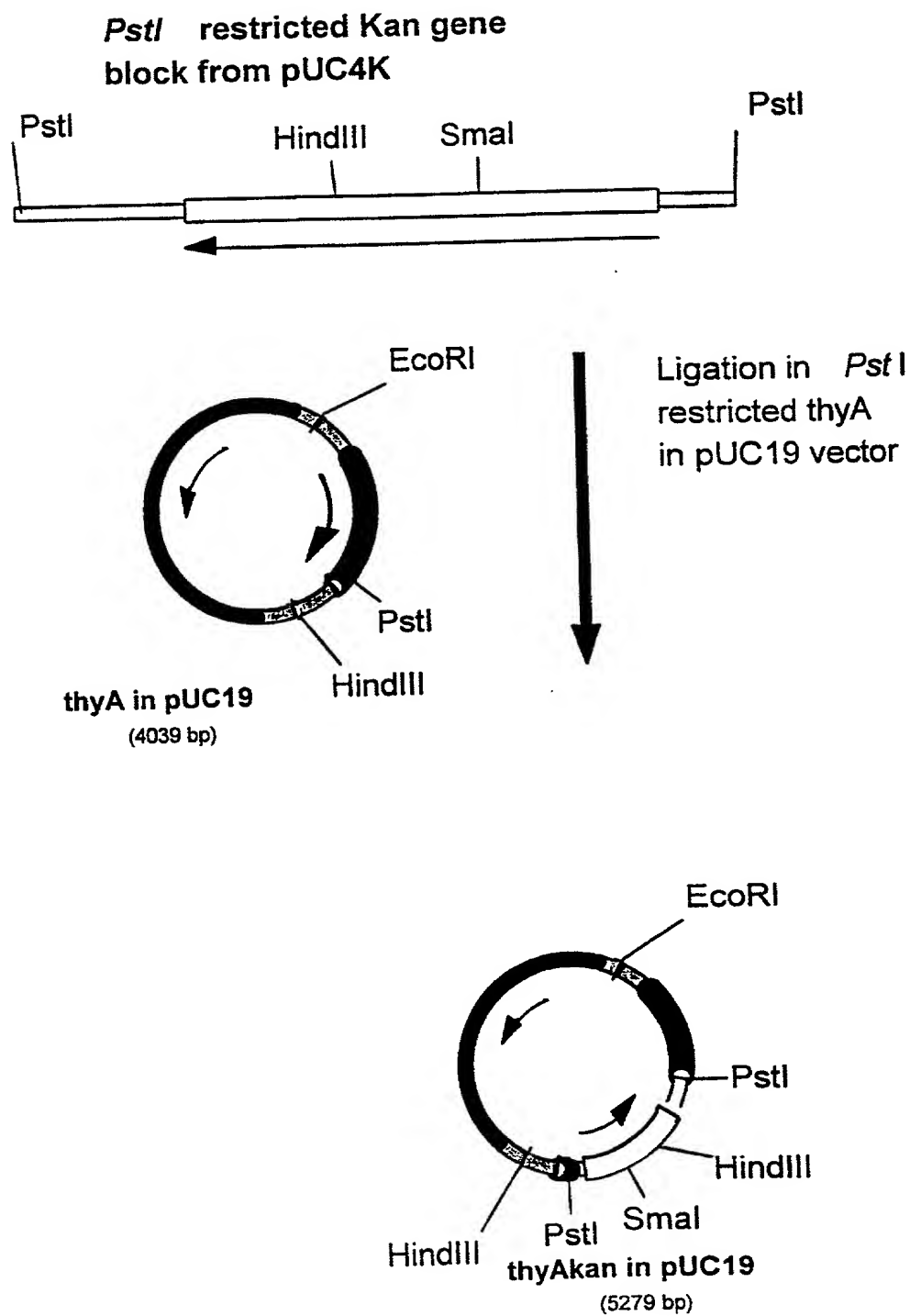
E. coli      : PLPKLIHKRPESIFYD----RFDFEIEGYDPHPGIKAPVAI
               | ::|::|::|      :|::|::|::|::|::|::|
V. cholerae  : PAPQFHINPKIKTLQDLETWVTLDDFDVTGYQFHDPIQYPFSV
               | ::|::|::|::|::|::|::|::|::|::|::|::|
H. influenza: PLPKELINPDIKTLEDLETWVTMDDFKVVGYQSHEPIKYPFSV

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FIG. 8



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FIG. 9

PCR to generate thyA-Kan-thyA fragment with *Xba*I ends.
 Primers were chosen so that the *Eco*RI and *Hind*III sites
 were eliminated

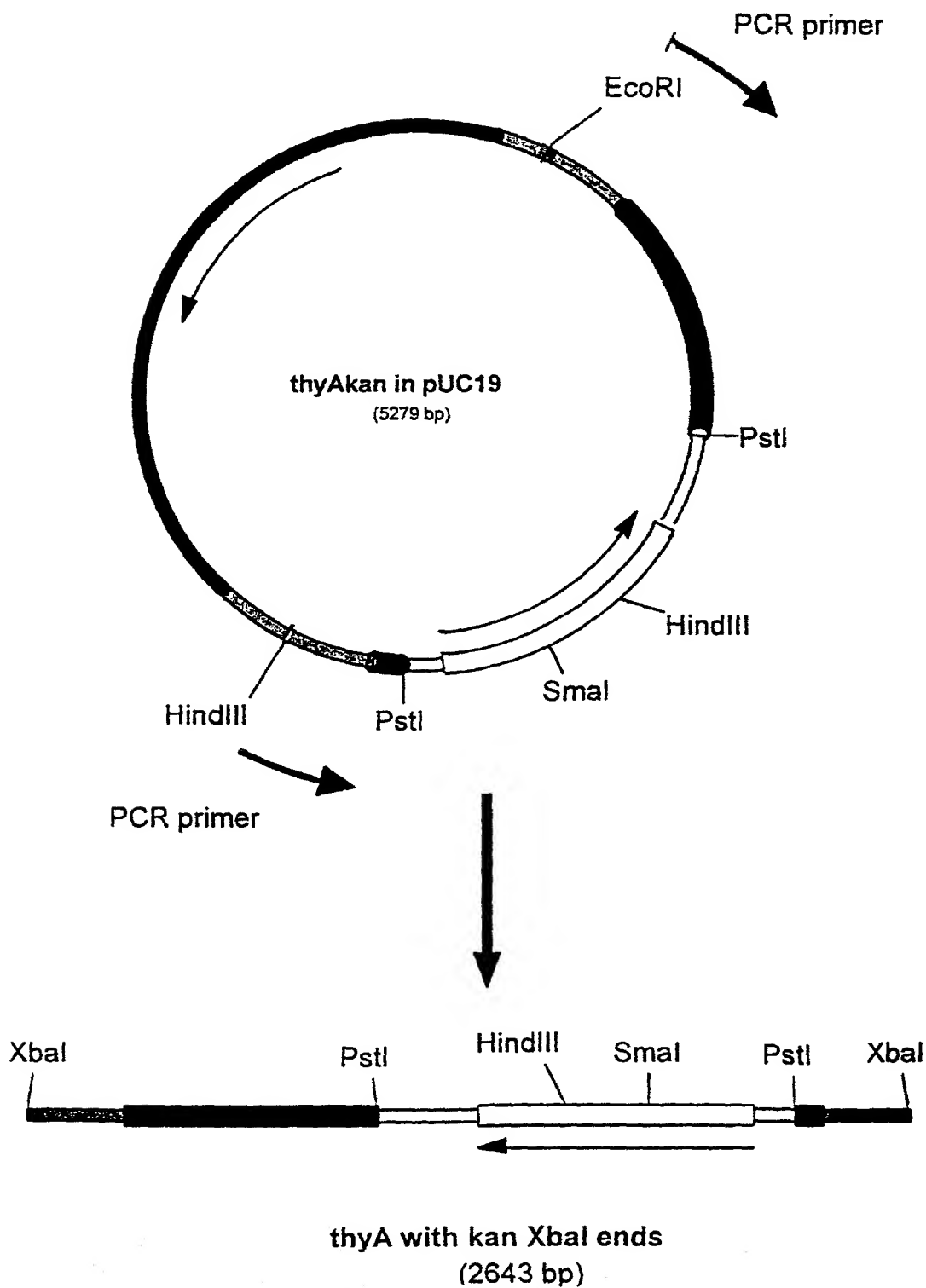
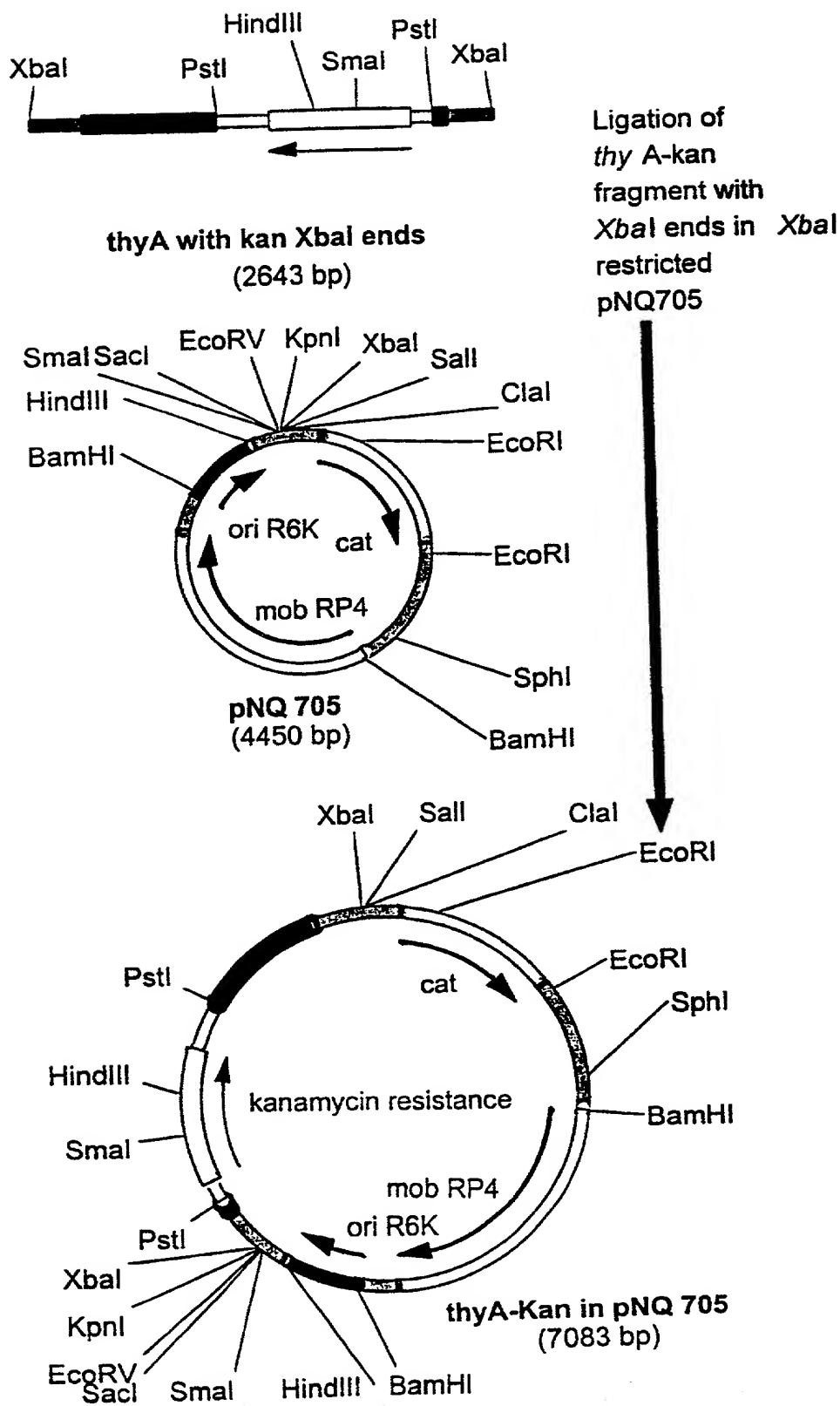


FIG. 10



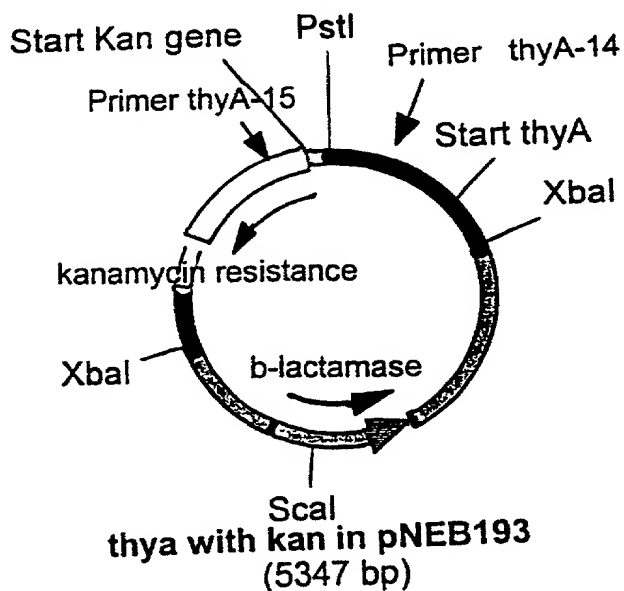
09/700712

PCT/EP99/03509

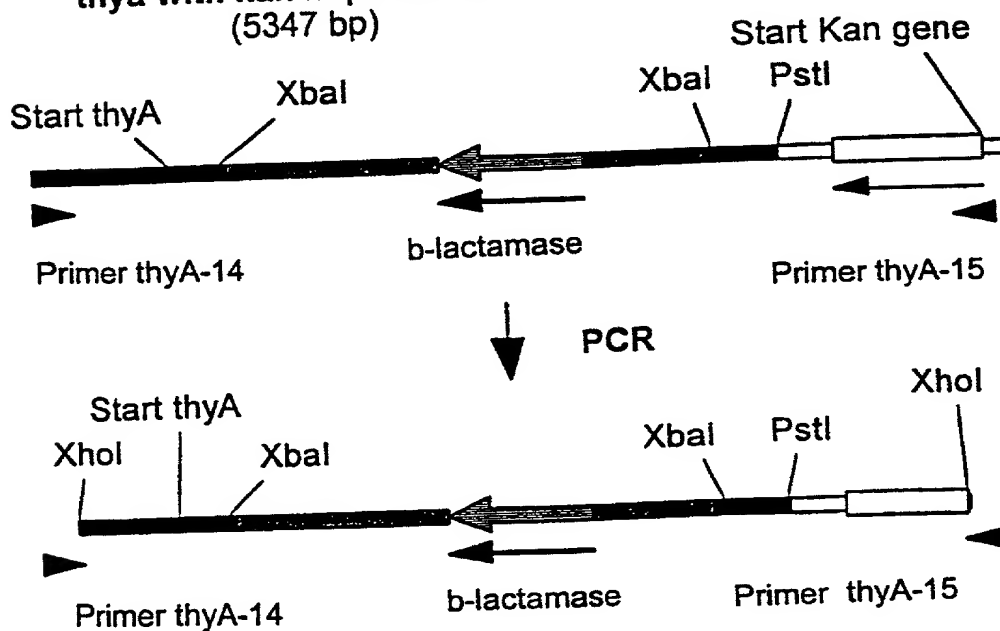
WO 99/61634

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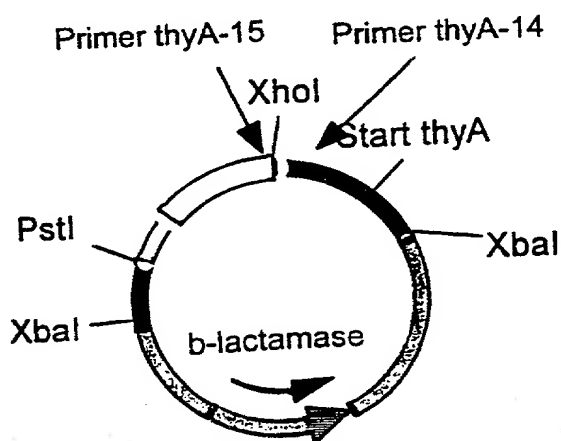
FIG. 11



PCR used to delete part of the *thyA* gene and the start of the Kanamycine resistance gene



PCR



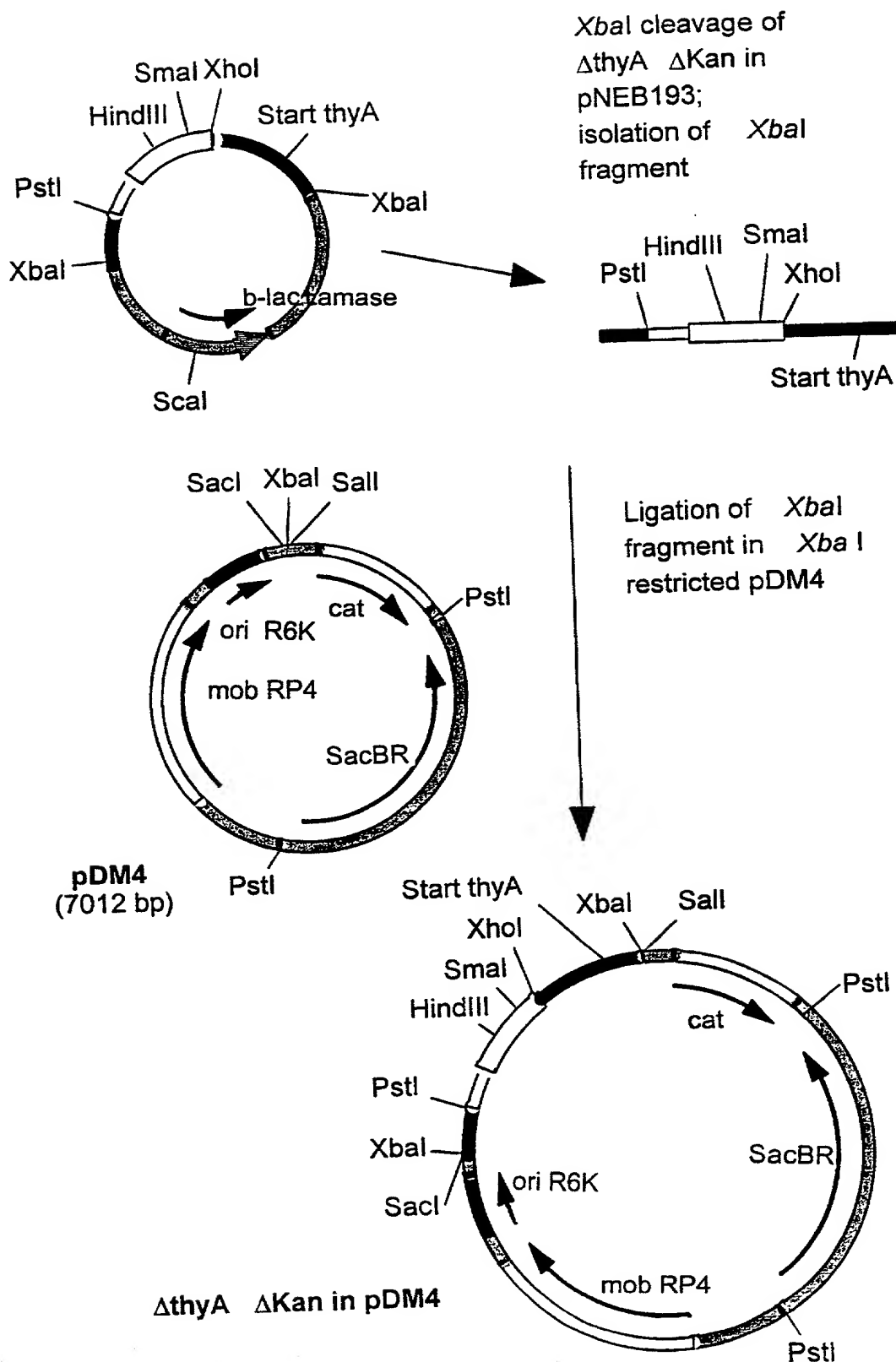
Cut with *XhoI* and self ligation

Δ *thyA* Δ *Kan* in pNEB193 (4877 bp)

SUBSTITUTE SHEET (RULE 26)

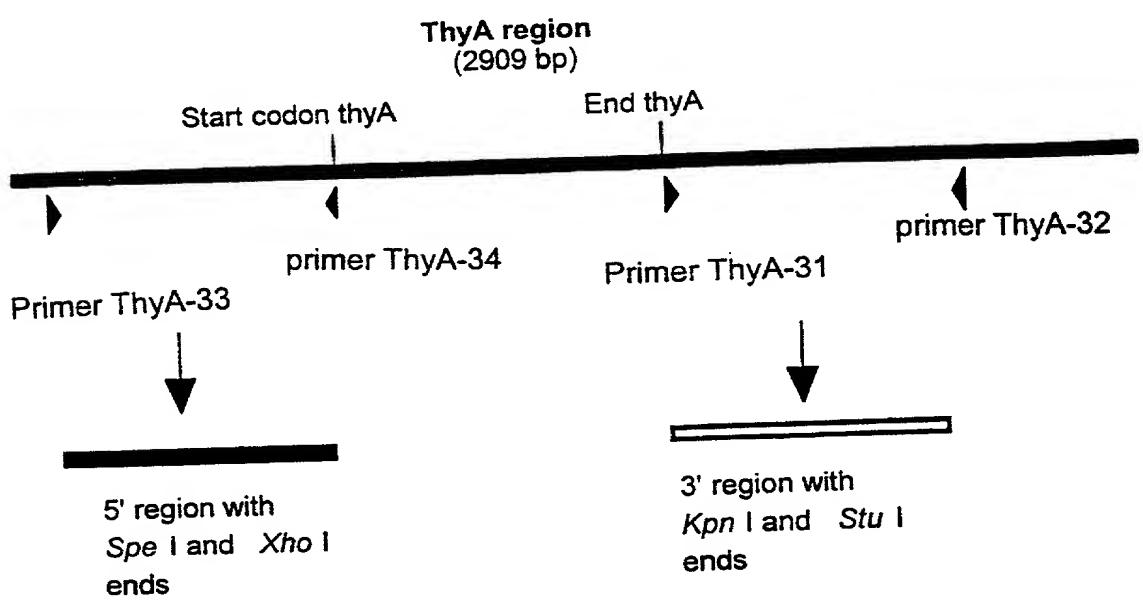
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FIG. 12



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FIG. 13



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FIG. 14

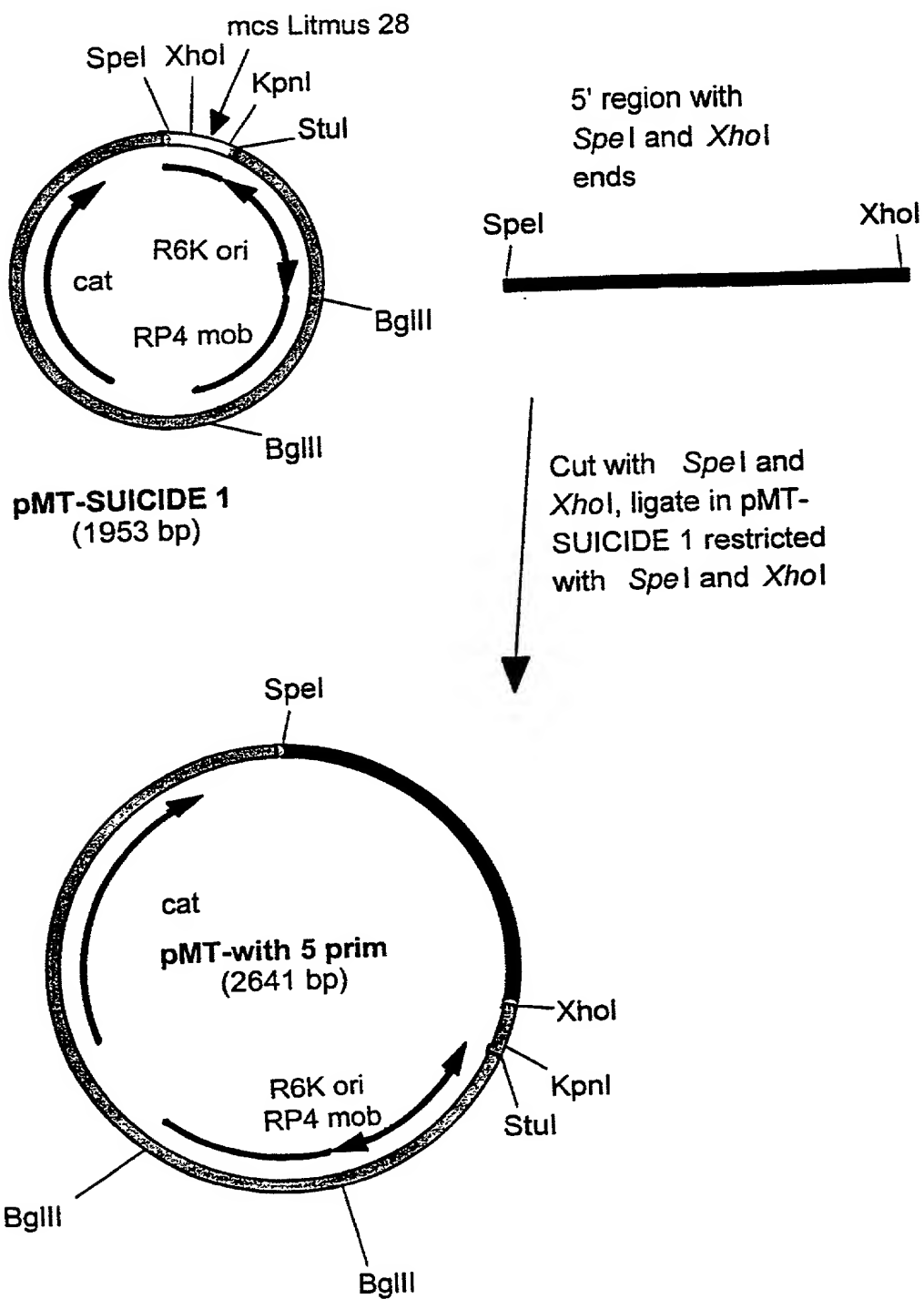
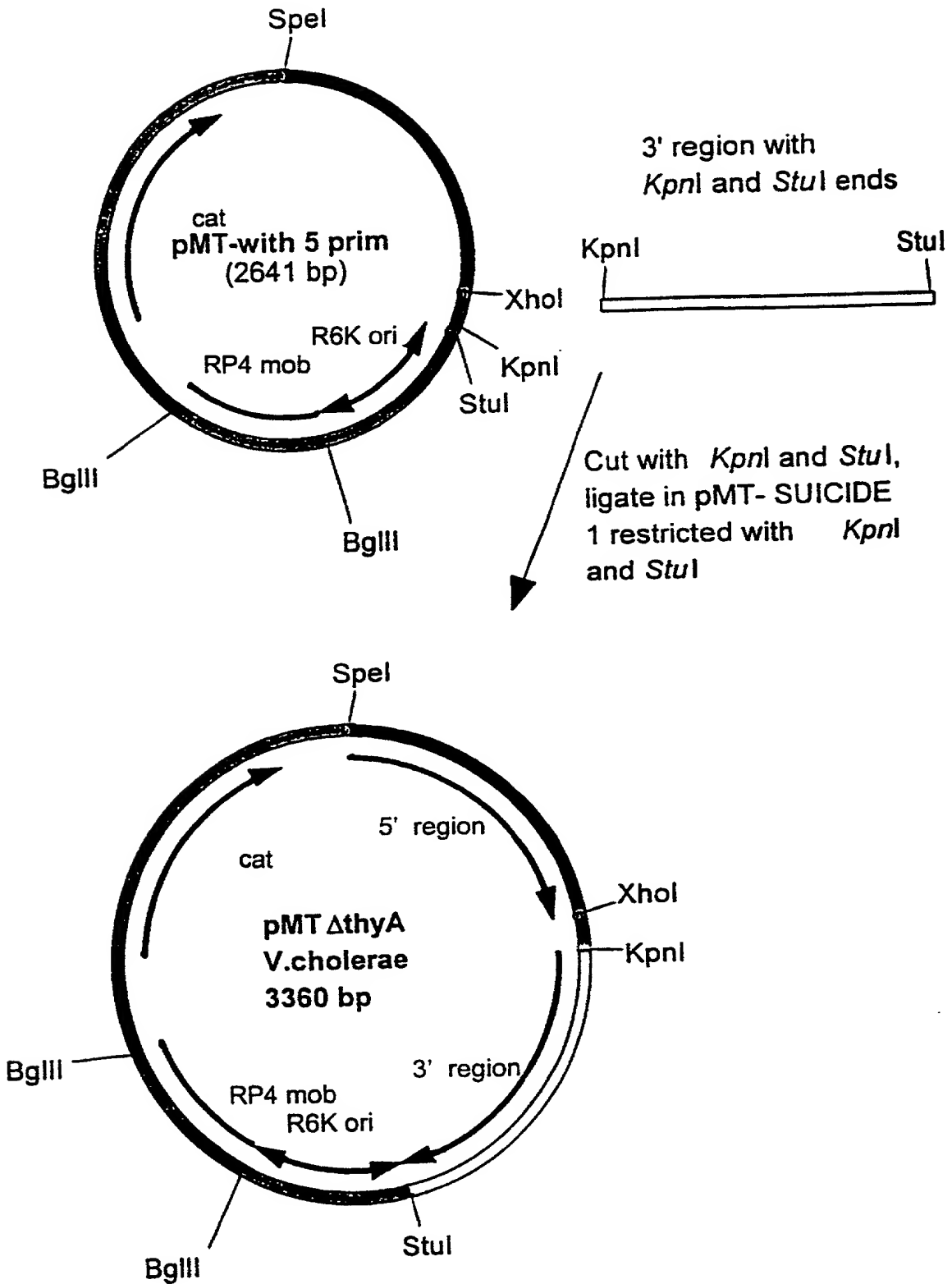


FIG. 15



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FIG. 16

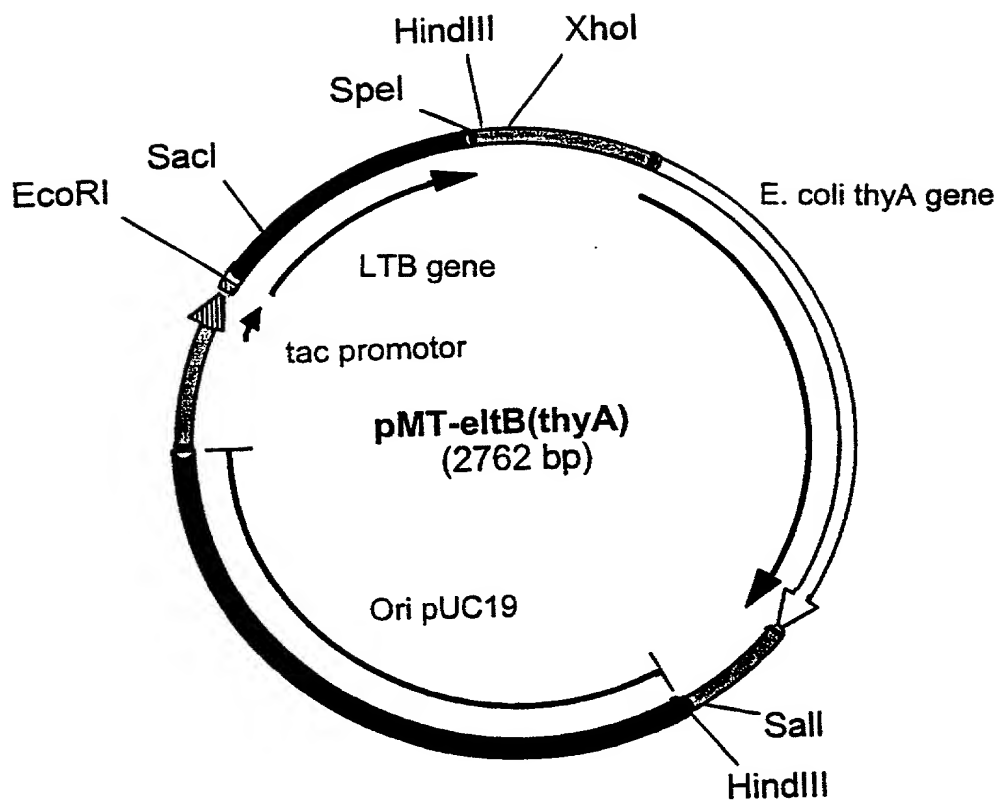


FIG. 17

